11:38:00 OCA PAD INITIATION - PROJECT HEADER INFORMATION 08/31/89

Active

Project #: G-33-616 Cost share #: Rev #: 0
Center # : 10/24-6-R6018-4A0 Center shr #: OCA file #:
Work type : RES
Contract#: 5 RO1 GM36610-05 Mod #: Document : GRANT
Prime #: Contract entity: GTRC

Subprojects ? : N
Main project #:

Project unit: CHEM Unit code: 02.010.136
Project director(s):
SUDDATH F L JR CHEM (404)894-4028

Sponsor/division names: DHHS/PHS/NIH / NATL INSTITUTES OF HEALTH
Sponsor/division codes: 108 / 001

Award period: 890801 to 900731 (performance) 901031 (reports)

Sponsor amount	New this change	Total to date
Contract value	171,182.00	171,182.00
Funded	171,182.00	171,182.00
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: HIGH RESOLUTION STRUCTURES OF PISUM SATIVUM LECTIN

PROJECT ADMINISTRATION DATA

OCA contact: Kathleen R. Ehlinger 894-4820

Sponsor technical contact

DR. JOHN NORVELL
(301)496-7260
NATL INST GENERAL MEDICAL SCIENCES
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BETHESDA, MD 20892

Sponsor issuing office

ANN CALURE
(301)406-7166
NATL INST GENERAL MEDICAL SCIENCES
RM 938 WESTWOOD BLDG
BETHESDA, MD 20892

Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : N/A N/A supplemental sheet
Equipment title vests with: Sponsor GIT X

Administrative comments -
INITIATION OF PROJECT.

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 08/02/90

roject No. G-33-616 Center No. 10/24-6-R6018-4A0_

roject Director SUDDATH F L JR School/Lab CHEMISTRY_____

ponsor DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH_____

ontract/Grant No. 5 RO1 GM36610-05_____ Contract Entity GTRC

rime Contract No. _____

itle HIGH RESOLUTION STRUCTURES OF PISUM SATIVUM LECTIN_____

ffective Completion Date 900731 (Performance) 901031 (Reports)

loseout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	_____
Final Report of Inventions and/or Subcontracts	N	_____
Government Property Inventory & Related Certificate	N	_____
Classified Material Certificate	N	_____
Release and Assignment	N	_____
Other _____	N	_____

Comments_____

_____

ubproject Under Main Project No. _____

ontinues Project No. _____

istribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other _____	N
_____	N

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G-32-616

SECTION IV PROGRESS REPORT SUMMARY	GRANT NUMBER GM36610-06	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Dr. F. L. Suddath	PERIOD COVERED BY THIS REPORT FROM 08/01/90	THROUGH 07/31/91
APPLICANT ORGANIZATION		

TITLE OF PROJECT *(Repeat title shown in item 1 on first page)*
High Resolution Structure of Pisum Sativum Lectin
(SEE INSTRUCTIONS)

1. There are no changes in the objectives of the proposal for next year.

2. The structure of the pea lectin dimer has now been determined to 1.7 Å resolution. The model has been refined against a new data set extending to 1.7 Å resolution collected using a rotating anode source and an area detector. Initially, the model was determined and refined to 1.9 Å resolution using data collected from a synchrotron source. The new model used the 1.9 Å model as a starting model for refinement against the area detector data. Both PROLSQ[1] and X-PLOR[2] were used in the refinement of the model. The current model at 1.7 Å resolution gives a crystallographic R value of 17.7 and an RMS deviation from ideal bond distances of 0.027 Å. At 1.7 Å resolution, atoms may be oriented with a high degree of confidence, giving a RMS error in coordinate positions (given by the Cruickshank method) of 0.125 Å.

The pea lectin molecule consists of two identical monomers that are related by a non-crystallographic two-fold axis to form a dimer. Each monomer consists of two chains, a b-chain, comprised of the N-terminal 181 amino acid residues, and an a-chain, made up of the C-terminal 47 amino acid residues. The two chains arise during processing of pea lectin, where a segment of several amino acid residues is enzymatically removed. As a result of this processing, the actual C-terminus of the b-chain is somewhat unclear. Protein sequencing[3] indicates that the C-terminus of the b-chain is Tyr-179. However, the electron ensity in this region clearly indicates the presence of Pro-180 and Asn-181 in both monomers. Recent evidence from HPLC experiments appears to support the presence of Asn-181[4]. During early stages of refinement, Pro-180 and Asn-181 were not included in the refinement model to avoid bias in the interpretation of the maps; however, density consistent with these residues continued to appear in the electron density maps, and both residues were eventually added to the model.

The model can now be analyzed with respect to the non-crystallographic two-fold symmetry. Deviations from perfect two-fold symmetry are observed which can be explained by crystal packing effects. Other differences are apparent upon examination of the temperature factors for corresponding residues in the two monomers. The average temperature factor for the B monomer (16.3 $Å^2$) is higher than that for the A monomer (14.5 $Å^2$). If all non-H_2O symmetry contacts are taken into account, the A monomer makes approximately 1.5 times as many contacts as the B monomer. Superposition of plots of average temperature factor versus residue number for the A and B monomers indicatess that the overall shape of the plots are very similar. Large differences arise near the C-terminus of the b-chains, where differences in contacts exist, and around residue 30 in the b-chains, where density has always been weak and modeling difficult, apparently due to disorder in this region.

Another interesting feature is the indication by the electron density map that several side chains, particularly threonines, leucines, isoleucines, and serines, exist in at least two major conformations, attained by rotation about c_1 (for leucine and serine) or c_2 (for threonine and isoleucine).

The electron density has also suggested positions for a number of bound water molecules, and, to date, 294 of these have been added to the refinement model. Eight of the water molecules are ligands to the two Mn^{2+} and two Ca^{2+} ions, completing the octahedral geometry around the ions. The ligand distances and angles around the metal ions is practically identical in the two monomers.

Nine water molecules were found to lie in the interface between the two monomers. One water molecule lies on the noncrystallographic two-fold axis, while the other eight form four symmetry related pairs. The water molecules in the interface form hydrogen bonds across the interface, bridging distances that would otherwise be too great. Most of the interactions forming the interface appear to involve hydrogen bonding, rather than hydrophobic interactions. This differs from the lectin Concanavalin A, which has a less hydrophilic interface and apparently no interface water molecules[5,6]. The implications of the predominately hydrophilic interface with regard to the possible function of pea lectin is not clear, nor is it clear why the pea lectin interface would differ from Con A, given that their functions are likely similar.

The remainder of the water molecules form hydrogen bonds to polar and charged side chains on the surface of the pea lectin dimer, and many are related by the two-fold symmetry of the dimer. Some of these surface water molecules lie near the putative carbohydrate binding site. Presumably, upon binding of a carbohydrate, the hydrogen bonds to water could be displaced by new hydrogen bonds to the hydroxyl groups of the carbohydrate molecule.

The coordinates of the pea lectin model at 1.7 Å resolution have been submitted to the Protein Data Bank[7].

Pea lectin is also being studied from a theoretical point of view using the technique of molecular dynamics. This computationally intensive technique allows one to study the motion of the atoms at a level not accessible by experiment. Essentially, one needs a starting atomic model (the x-ray coordinates in this case), and an empirical potential energy function. From the potential energy function, one can then calculate the forces on each atom. By integrating Newton's equations of motion, that is, allowing each atom to move in the direction of its force, one obtains a series of snapshots of the molecule at different times. Examination of the dynamical nature of the protein can lead to a great understanding of what types of motion are available and functionally important to the protein.

Several simulations are being carried out in this laboratory. For the most part, these calculations are being performed using the molecular dynamics program CHARMm running on a Silicon Graphics Compute Server 240S and visualized on a Silicon Graphics Iris 4D/70 GT Workstation. First, a 100 picosecond (ps) simulation of the protein in vacuo (no explicit water molecules present) has been done. Preliminary analysis shows large deviations from the x-ray model occur where the density is weak Also, simulations are being performed where the crystallographic waters are included explicitly, as well as a simulation using 3 Ångstrom solvent shell around the surface of the water. These waters were restrained to their original positions using a weak quartic potential. Lastly, a simulation of pea lectin in a box of water molecules using periodic boundary conditions, will be performed providing the most realistic model of the protein. These calculations take considerably longer not only because of the increased number of atoms, but because the equilibration phase of the calculation is also increased. These simulations will help elucidate the structural role that the water molecules play in the stability of the protein.

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[1]Hendrickson, W.A. and Konnert, J.H. (1979) In *Biomolecular Structure, Conformation, Function, and Evolution.* (R. Srinavason, Ed.) Pergamon Press, New York, vol I, pp 43-47.

[2]Brunger, A.T. (1988) X-PLOR Manual. X-PLOR software (1987) President and Fellows of Harvard University.

[3]Rini, J.M., Hofmann, T., and Carver, J.P., unpublished data.

[4]Jackson, G.E.D. and Young, N.M. (1987) *Anal. Biochem.* **162**, 251-256.

[5]Edelman, G.M., Cunningham, G.A., Reeke, G.N. Jr., Gecker, J.W., Waxdal, M.J., and Wang, J.L. (1972) *Proc. Natl. Acad. Sci USA*, **69**, 2580-2584.

[6]Harkman, K.D. and Ainsworth, C.F. (1976) *Biochemistry* **11**, 4910-4919.

[7]Bernstein, F.C., Koetzle, T.F., Williams, G.J.B., Meyer, E.F. Jr., Brice, M.D., Rodgers, J.R., Kennard, O., Shimanouchi, T., and Tasumi, M. (1977) *J. Mol.Biol.* **112**, 535-542.

CHECKLIST	GRANT NUMBER GM36610-06

Check the appropriate boxes and provide the information requested. Make this page the last page of the signed original of the application. **Do not attach copies of this page to the duplicated copies of the application.**

ASSURANCES

The following certifications described below are made by checking the appropriate boxes and verified by the signature of the OFFICIAL SIGNING FOR APPLICANT ORGANIZATION on the FACE PAGE of the application.

a. **Delinquent Federal Debt.** ☑ No ☐ Yes (If "Yes," attach explanation.)

Before a grant award can be made, the applicant organization must certify that it is **not** delinquent on the repayment of any Federal debt. The certification applies to the applicant organization, **not** to the person signing the application as the authorized representative **nor** to the principal investigator/program director.

Examples of Federal debt include delinquent taxes, audit disallowances, guaranteed or direct student loans, FHA loans, business loans, and other miscellaneous administrative debts. For purposes of this certification, the following definitions of "delinquency" apply:

- **For direct loans and fellowships** (whether awarded directly to the applicant by the Federal Government or by an institution using Federal funds), a debt more than 31 days past due on a scheduled payment. (Definition **excludes** "service" payback under a National Research Service Award.)
- **For guaranteed and insured loans,** recipients of a loan guaranteed by the Federal Government that the Federal Government has repurchased from a lender because the borrower breached the loan agreement and is in default.
- **For grants**, organizations in receipt of a "Notice of Grants Cost Disallowance" which have not repaid the disallowed amount or which have not resolved the disallowance. (Definition **excludes** disallowances in an "appeal" status.)

Where the applicant discloses delinquency on debt to the Federal Government, the PHS shall (1) take such information into account when determining whether the prospective grantee organization is responsible with respect to that grant, and (2) consider not making the grant until payment is made or satisfactory arrangements are made with the agency to whom the debt is owed. Therefore, it may be necessary for the PHS to contact the applicant before a grant can be made to confirm the status of the debt and ascertain the payment arrangements for its liquidation. Applicants that fail to liquidate indebtedness to the Federal Government in a businesslike manner place themselves at risk of not receiving financial assistance from the PHS.

b. **Debarment and Suspension.** ☑ No ☐ Yes (If "Yes," attach explanation.)

Before a grant award can be made, the applicant organization must certify, among other things, that neither it nor its principals are presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency. Subawardees, that is, other corporations, partnerships, or other legal entities (called "lower tier" participants), must make the same certification to the applicant organization concerning their covered transactions. Please refer to the pertinent DHHS implementing regulations, Title 45 Code of Federal Regulations Part 76, for complete certification requirements.

c. **Drug-Free Workplace.** ☑ Yes ☐ No (If "No," attach explanation.)

Before a grant award can be made, the applicant organization must certify that it will provide a drug-free workplace. The main points of the certification require the applicant organization to:

- Publish a statement notifying employees that the unlawful manufacture, distribution, dispensation, possession, or use of a controlled substance is prohibited in the workplace and specifying the actions that will be taken against employees for violation of such prohibition;
- Establish a drug-free awareness program;
- Require that each employee engaged in the performance of a grant or contract be provided a copy of the published statement;
- Notify the employee that as a condition of employment, the employee will abide by the terms of the statement;
- Notify the PHS awarding component of any employee convicted of a drug violation occurring in the workplace; **and**
- Require any employee who is convicted of a drug offense occurring in the workplace to participate in a rehabilitation program.

Please refer to the pertinent DHHS implementing regulations, Title 45 Code of Federal Regulations Part 76, for complete certification requirements.

INDIRECT COST CALCULATION

Indicate the applicant organization's most recent indirect cost rate established with the appropriate DHHS Regional Office, or, in the case of for-profit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office. Indirect costs will not be paid on foreign grants, construction grants, grants to Federal organizations and grants to individuals, and usually not on conference grants. Follow any additional instructions provided for Research Career Development Awards, Institutional National Research Service Awards, and specialized grant applications.

☐ DHHS Agreement Dated: _____ ☐ No Indirect Costs Requested

☒ No DHHS Agreement, but rates established with Office of Naval Res. DATE 04/13/89

***CALCULATION**

Enter proposed budget period:

Amount of Base $ 119,985 (less equipment) 113,485 x Rate Applied .625 % = Indirect Costs $ 70,928

Add to total direct costs from page 2 and enter new total on FACE PAGE, Item 10b

*Check appropriate box(es)

☐ Salary and wage base ☑ Modified total direct costs base ☐ Other base (Attach explanation)

☐ Off-site, other special rate, or more than one rate involved (Attach explanation)

*This is the required last page of the application.